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(Sutochnaya periodichnost' stadii spermatogeneza severnogo olenya)

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DIURNAL PERIODICITY OF SPERMATOGENESIS STAGES IN REINDEER
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E.K. Borozdin

The existence of a diurnal rhythm in the mitotic activity of most animal tissues is at present well established. Scientists of all countries have in the main been attracted by problems of diurnal mitotic dynamics of such organs as cornea, epidermis, liver, etc. But we have not found in the available literature any investigations of the diurnal periodicity of stages of spermatogenous epithelium activity. Yet, study of this topic may well result in deeper understanding of the physiology of reproductive organs, the determination of rhythms occurring in meiosis, and the application of these new insights to methods of invigorating sexual function, which is of considerable importance in animal breeding.

This paper investigates diurnal mitosis dynamics and the basic stages of spermatogenesis in reindeer. The processes were studied under natural conditions.

THE MATERIAL AND METHOD

Experimentation was carried out at the Potapov experimental breeding farm of the institute (Taimyr National District). A group of male animals (16 head), one and a half years old, was used. They were castrated, two at a time, every 3 hours in the course of a 24-hour period. The experiment took place on 10 September (some 10 - 12 days before the onset of rut, during the period of highest spermatogenetic activity). Histological material was in every instance taken from the same section of testicle. Fixation was carried out by the Carnoi method. Sections, 7 microns in thickness, were stained with hematoxylin by the Heidenhein method. For each animal the number of cells in each phase of spermatogenesis was counted in cross sections of 10 convoluted tubules. The stages of spermatogenesis were determined by observing 100 tubules without a cell count, in order to determine the mutual interrelationship of spermatogenetic waves. Mitotic activity was determined according to the number of cells in the metaphase, anaphase and telophase. Mitotic activities of A and B spermatogonia were counted together. Statistical reliability was determined by using the Student table. In addition, we put the animals at pasture under observation for a 12-hour period to time the sexual activity of the males.

TABLE 1. Quantity of cells by stages of spermatogenesis at different hours of day and night

Hours of castration	Quantity of cells of stages of spermatogenesis, %												
	spermatogonia			spermatocytes of the first order						spermatocytes of the second order		spermatids	spermato-zoids
	A	B	meta-, ana- and telophase	early pro-phase	leptonema	synapsis	pachynema	diakinesis	meta-, ana- and telophase	prophase	meta-, ana- and telophase		
0	13.6 ±3.9	8.85±0.95	0.7 ±0.4	1.6 ±0.3	4.15±3.05	—	15.4 ±3.8	11.1 ±8.4	2.15±1.45	3.15±1.05	0.15±0	37.7 ±16.5	3.05±3.05
3	12.25±1.05	7.2 ±0.5	1.2 ±0	2.7 ±0.7	7.05±2.55	1.7 ±1.7	18.65±1.15	12.15±8.95	3.6 ±1.15	5.9 ±0	—	18.75±11.9	2.45±0.95
6	9.9 ±2.7	6.8 ±4.3	0.4 ±0.1	3.35±1.25	9.05±4.25	2.8 ±2.8	20.75±3.35	2.0 ±2.0	0.15±0.15	4.4 ±4.4	—	37.2 ±10.9	2.95±2.5
9	11.85±0.95	10.6 ±0.5	0.35±0.25	0.2 ±0.1	14.8 ±0.65	—	15.2 ±3.9	—	—	1.75±0.25	—	41.85±0.95	3.85±0.4
12	11.1 ±0.8	11.6 ±0.7	0.3 ±0.25	0.1 ±0.1	5.65±0.65	—	28.9 ±3.9	0.35±0.15	0.55±0.15	0.9 ±0.8	—	38.65±3.05	5.85±1.7
15	15.7 ±2.9	10.1 ±1.2	0.15±0.15	0.05±0.05	5.83±1.5	—	34.65±10.95	0.2 ±0.2	0.25±0.25	2.6 ±1.4	—	17.85±1.55	0.3 ±0.3
18	16.25±1.25	13.95±0.25	0.05±0.05	0.65±0.65	7.55±0.15	1.4 ±1.4	28.7 ±11.5	0.01±0	0.6 ±0.6	0.9 ±0.1	0.5 ±0.5	34.9 ±8.4	—
21	18.0 ±4.8	10.2 ±4.5	0.55±0.25	1.45±0.45	2.0 ±2.0	3.15±3.05	30.2 ±6.3	0.4 ±0.4	—	1.65±1.65	0.1 ±0.1	29.85±2.45	—
R	0.184	0.122	0.016	0.014	0.000	—	0.211	0.056	0.070	0.024			

RESULTS OF INVESTIGATIONS

The diurnal dynamics of the mitotic activity of spermatogonia presents a clearly marked character. The maximum quantity of dividing cells, both in absolute numbers (Table 1) and in relation to the number of tubules containing spermatogonia in the process of division (Table 2), was observed at 0300 hours, and the minimum quantity at 1800 hours. At 0300 hours the number of mitoses is 24 times as great as at 1800 hours. Decrease and increase in mitotic activity take place gradually and uniformly.

TABLE 2.

Quantity of tubules at spermatogenesis stages at different hours of day and night

hours of castration	Quantity of tubules with spermatogenesis stages, %												
	spermatogonia			spermatocytes of first order						spermatocytes of second order		spermatids	spermatozoids
	A	B	meta-, ana- and telophase	early prophase	leptonema	synapsis	pachynema	diakinesis	meta-, ana- and telophase	prophase	meta-, ana- and telophase		
0	95±5	61±5	17±7	13±1	29±11	4±2	100±9	50±14	29±10	23±7	2±2	92±2	7±7
3	67±3	73±3	40±12	9±1	27±1	7±5	93±9	22±6	23±7	19±1	2±2	95±5	17±15
6	88±8	55±7	25±3	27±3	37±9	13±5	79±15	20±6	10±4	15±3	—	90±0	4±2
9	95±3	47±11	11±3	19±5	35±15	2±2	79±11	14±6	11±7	22±10	3±1	100±0	4±2
12	91±7	46±10	13±1	13±1	25±5	5±5	99±7	11±1	10±2	16±16	5±1	99±1	11±9
15	100±0	64±10	7±3	6±4	26±8	—	100±17	14±14	7±3	21±5	—	98±1	3±3
18	93±1	73±7	4±4	13±5	33±1	5±3	81±4	15±9	19±9	11±3	3±1	98±2	1±1
21	97±1	68±8	9±1	15±3	21±7	6±6	100±12	7±1	15±1	7±1	1±1	94±6	4±4
R	0.007	0.023	0.009	0.070	0.241	0.161	0.747	0.061	0.027	0.036			

An insignificant increase of activity was observed at 1200 hours, as the result of division of A-type spermatogonia. During the nighttime the main mass of dividing cells is of the B-type spermatogonia

In the dynamics of spermatogonia content, certain regularities are also observed. The smallest number of A-type spermatogonia was found in the period between 0300 and 0600 hours, and the greatest number between 1200 and 1800 hours, which corresponds to the already noted increase in the mitoses of A-type spermatogonia at 1200 hours. It should be observed that division of A-type spermatogonia again brings about the formation of A-type spermatogonia We have not succeeded in observing formation of B-type spermatogonia, but in all probability it does take place. B-type spermatogonia reach maximum quantity at 1800 hours, i. e., at the period of lowest mitotic activity of spermatogonia; and as this activity increases, the quantity gradually becomes smaller.

The diurnal pattern in the quantity of spermatocytes of the first order in early prophase corresponds to the pattern of spermatogonia mitoses. The peak numbers of spermatocytes of the first order in early prophase appear immediately after the highest mitotic spermatogonia activity, i.e., at 0600 hours. Thereafter, the numbers of spermatocytes of the first order in early prophase stage gradually decrease, reaching a minimum at 1500 hours.

The quantity of spermatocytes of the first order in the leptotene stage and the percentage of tubules containing this generation of cells show a fairly clear pattern of diurnal dynamics, with the maximum at 0900 hours, and the minimum at 2100 hours. But the total percentage of spermatocytes in leptotene is much higher than in early prophase. Apparently, the cells of this generation accumulate because they remain in this stage for a longer period.

There is no clearly marked diurnal periodicity in the synapsis and pachynema stages, especially in relation to the quantity of cells. One may only point out a certain tendency in pachynema toward an increased quantity of cells during the second half of the day, i.e., from 1500 to 2100 hours. At all other hours of the day and night the quantity of cells in these stages remains more or less constant with only slight and uneven fluctuations.

The diakinetic stage immediately precedes the first maturation division and corresponds to the diurnal mitotic activity of spermatocytes of the first order. In diakinesis the quantity of cells is subject to very considerable fluctuation. The maximum quantity of cells in this stage is 121 times as great as the minimum and constitutes up to 12% of the total number of cells. In diakinesis, as in dividing spermatocytes of the first order, cells of this generation are found in the maximum number of tubules at 2400 hours, but invariably in comparatively small numbers in each tubule. At 0300 hours the number of tubules containing spermatocytes decreases, while the cells in this stage in each tubule increase. In all likelihood cell division in many of the tubules occurs during the period from 0000 to 0300 hours.

The highest mitotic activity of spermatocytes of the first order is observed from 2400 to 0300 hours. By 0600 hours the quantity of dividing cells dwindles to one eighteenth of the maximum, and the number of tubules exhibiting mitoses is 2.5—3 times smaller, indicating a very fast cell division. In the ensuing hours the percentage of cells in the process of first maturation division fluctuates between 0% and 0.6%. No even rise or fall in the number of tubules containing dividing spermatocytes of the first order was observed. For the most part such tubules amount to about 7—10% but at 1800 hours their number suddenly rises to 18%, and by 2100 hours drops again to 5%. No coincident increase could be noticed in the quantity of cells in the first maturation-division stage.

Regular patterns are shown by the quantity of spermatocytes of the second order, corresponding to the dynamics of the first maturation division, but with considerably smaller flunctuations. Thus, at 0300 hours the percentage of cells in this stage is only 6.5 of that at 1500 hours (cells in the prophase, metaphase and anaphase stages counted together). The number of cells declines and rises fairly evenly and gradually. No uniform diurnal dynamics was detected in the quantity of tubules containing this generation of cells, nor in the division of spermatocytes of the second order. Apparently, the second maturation division runs its course so fast as to be almost incapable of detection, and the small quantity of observable

cells does not warrant any conclusions as to the existence of a diurnal uniformity. The diurnal variations in the quantities of cells and tubules in the second maturation division, as observed in our investigations, were accidental in character.

Spermatids were encountered in practically all tubules. No diurnal periodicity could be seen in the total quantity. This matter requires separate study, with classification of spermatids by the stages of their transformation into spermatozoa. And the same may be said of spermatozoids. We only took note of the quantity of spermatids and spermatozoa, and of the number of tubules in which they were found with a view to obtaining a general description of the spermatogenesis and a determination of the interrelationship of its waves.

Survey of DNA quantity found in the course of reindeer spermatogenesis shows that the DNA synthesis takes place at the B3 and B4 spermatogonia stages, and also during the first two stages of spermatocytes of the first order, i.e., in early prophase, apparently incorporating the interphase and, it seems, also during the leptotene. The greatest number of cells in these stages is recorded at night: the B3 and B4 spermatocytes from 2100 to 0300 hours; spermatocytes of the first order in early prophase from 2400 to 0600 hours; and those in leptotene from 0600 to 0900 hours. These data confirm the opinion of an entire group of authors /1, 6/ that inhibition of mitotic activity and the diurnal periodicity of cell division are related to nuclein metabolism, which apparently plays an essential part in these processes.

The determination of stages of spermatogenesis in the tubules has shown that at different hours in the course of the day and night diverse associations of spermatogenous cells predominate. This determination of spermatogenetic waves by cell combinations in the tubules /1/ warrants the conclusion that the movement of cells along the spermatogenetic wave is closely related to the diurnal periodicity of spermatogenesis stages and, in its turn, displays a consistent diurnal dynamics.

The observation of stags' behavior at pasture during the mating period showed that the highest degree of sexual activity of the males of the species occurs during the morning hours, especially in the pre-dawn period. The intensity of rut gradually subsides after that, growing stronger again in the evening at sunset. Sexual activity of deer during nocturnal hours is minimal. Variations in the length of the day and night are reflected in corresponding variations of these spans of sexual intensity. No connection has come to light between the diurnal rhythms of spermatogenetic stages and the sexual activity of males of the species.

The majority of investigators / 2 - 8 / who have considered the influence of various factors on the mechanism of diurnal periodicity in mitoses offer various explanations of this process. Examination of the influence of diverse factors on the diurnal dynamics of spermatogenetic stages is not the object of the present work. We concur in the opinion, expressed by I. A. Alov / 2 /, that the diurnal dynamics of mitotic activity is associated with photoperiodism and with the functional mechanics of the relevant body part and, therefore, with the nervous system which regulates the processes involved. It seems, however, that the influence of these factors on cell multiplication is not a direct one. It is the nuclein metabolism and the duration of the prophase in which chromosome reconstructions play the principal part that are regarded by us as the intermediate links between the neurohumoral functional regulation of individual organs and the mitotic

activity of their component cells. According to the data supplied by G. S. Strelin and V. V. Kozlov / 7 / the transition of cells from interkinetic stage to mitosis ceases upon stimulation, but cells that have already commenced the process of division continue to divide normally. This indicates the participation of an inner automatism in the process of cell division. But it is difficult to explain the strict diurnal periodicity obtaining in the division of spermatocytes of the first order, which have a prolonged meiotic prophase, and of spermatocytes of the second order in which interkinesis does not occur at all.

Diurnal periodicity in the formation of sex cells is not a unique characteristic of spermatogenesis; the study of egg yield in birds shows that it also influences oogenesis / 8, 9 /.

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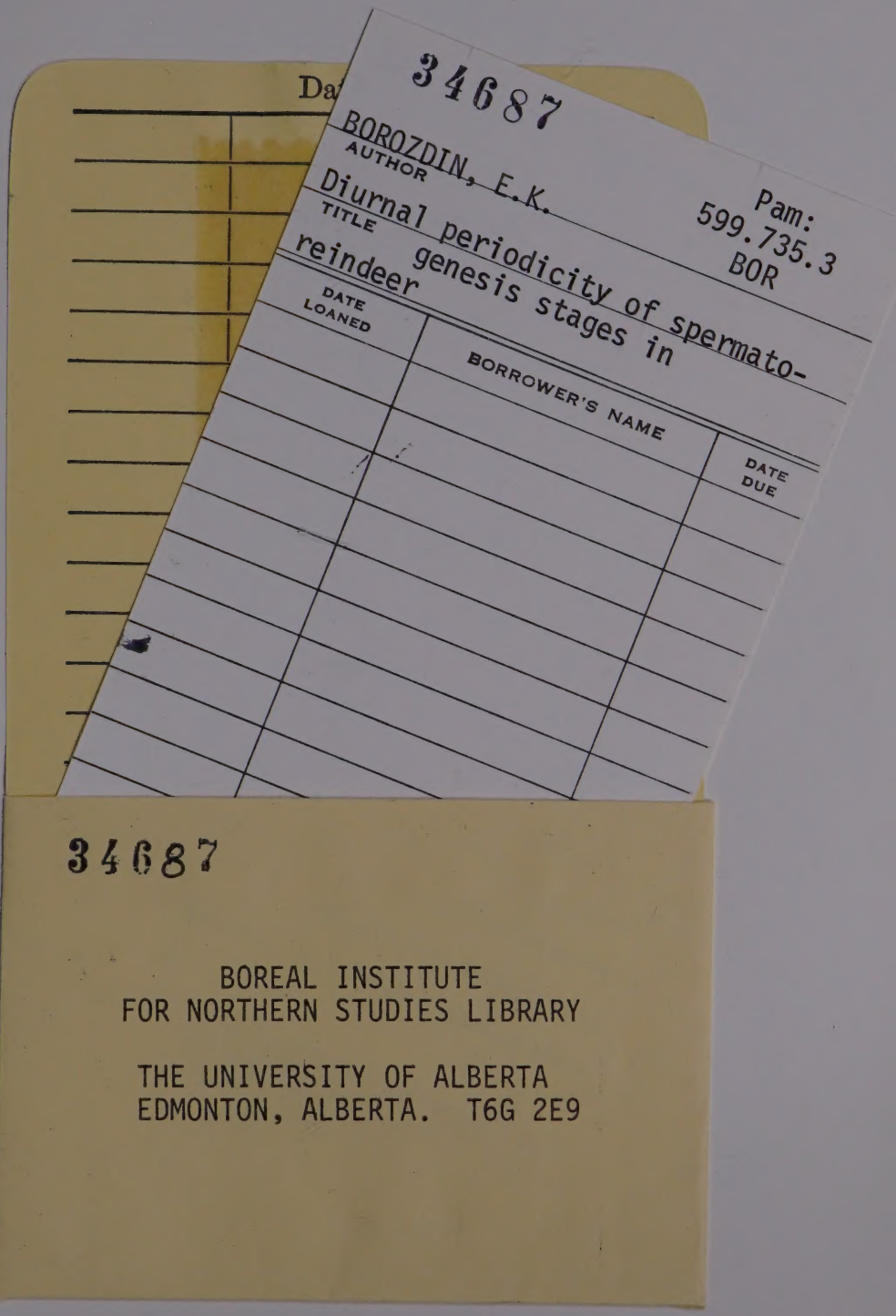
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